

AMENDMENTS

In the specification:

On page 30, please delete the paragraph on page 30, line 29, to page 31, line 16, and substitute therefor:

Cells were recovered and incubated with 0.5 mg/ml lysozyme in 50 mM Tris (pH 8.0), 150 mM NaCl, 1% TweenTM-20, 0.1% 2-mercaptoethanol, 5 mM EDTA, 1 mM PMSF and a mixture of other protease inhibitors obtained from Boehringer Mannheim (1697498) at room temperature for 0.5 h, followed by sonication. Cellular debris were pelleted by centrifugation at 27,500 g for 10 min and the resulting supernatants were incubated with 30 ml of glutathionine-SepharoseTM (Pharmacia) at 4° C overnight. The resin was then washed with 20 mM Tris (pH 8.0), 150 mM NaCl, 0.1% TweenTM-20, and 0.1% 2-mercaptoethanol until the OD 280 nm reached <0.01. For removal of GST, the resin with immobilized GST-fusion protein was incubated with 10U of thrombin (Boehringer, Inc.) at 4° C in 20 mM Tris (pH 8.0), 150 mM NaCl, 0.1% TweenTM-20, 0.1% 2-Mercaptoethanol, and 2.5 mM CaCl₂ overnight. Released proteins were then purified on Mono QTM (HR10/10, Pharmacia) by FPLC using a linear gradient of 0.5M NaCl at pH 8.0 and dialyzed into chaperone assay buffer.

On page 32, please delete the section header on lines 24-25 and substitute therefor:

C. BIAcoreTM Assay of BAG Protein Binding to the ATPase Domain of Hsc70

On page 33, please delete the paragraph on page 33, line 5, to page 34, line 8, and substitute therefor:

BAG-family proteins were produced in bacteria and purified to near homogeneity as shown in Figure 12A and described above in Example I. The purified BAG-1 (beginning at residue 116 of SEQ ID NO:2), -2 (SEQ ID NO:4), and -3 (SEQ ID NO:6) proteins were then immobilized on biosensor chips and tested for their interactions with Hsc70 in the soluble phase.

Kinetic measurements were performed using a BIAcoreTM-II instrument with CM5 sensor chip and Amine Coupling Kit (Pharmacia Biosensor AB, Sweden). Briefly, for immobilization of proteins, the sensor chip was equilibrated with HK buffer (10 mM Hepes (pH 7.4), 150 mM KCL) at 5 μ l/min, then activated by injecting 17 μ l of 0.2M N-ethyl-N'-(3-diethylaminopropyl)-carbodiimide and 0.05M N-hydroxysuccinimide (NHS/EDC) followed by 35 μ l of the protein of interest, in 10 mM acetate, pH 3.5-4.5. Excess NHS-ester on the surface was deactivated with 17 μ l 1M ethanolamine-HCL (pH 8.5). After immobilization, 5 μ l of regeneration buffer (50 mM phosphate (pH 6.8) and 4M GuHCl) was injected. For binding assays, Hsp70 (Sigma, H8778) was dissolved in HK buffer, and injected at 10 μ l/min across the prepared surface at various concentrations. The surface was regenerated after each injection with 5 μ l of regeneration buffer. The rate constants K_{ass} and K_{diss} were generated with BIAevaluation software software 3.01 (Pharmacia Biosensor AB). Addition of Hsc70 to chips containing BAG-1 (beginning at residue 116 of SEQ ID NO:2), BAG-2 (SEQ ID NO:4) or BAG-3 (SEQ ID NO:6) resulted in concentration-dependent binding, as reflected by an increase in the Response Units (RU) measured at the chip surface (shown in Figure 3B). In contrast, Hsc70 failed to display interactions in BIAcoreTM assays with a variety of control proteins as well as a mutant of BAG-1 lacking a C-terminal portion of the BAG domain which is required for Hsc70-binding (Figure 3B). Furthermore, flowing of various control proteins such as GST, BSA and Bcl-XL over the BAG-1 (beginning at residue 116 of SEQ ID NO:2), BAG-2 (SEQ ID NO:4), or BAG-3 (SEQ ID NO:6) chips resulted in negligible interaction. These results further demonstrate the specificity with which BAG-family proteins interact with and bind to Hsc70.

On page 36, please delete the paragraph on lines 14-30 and substitute therefor:

In an additional refolding assay, described previously by Minami et al., J. Biol. Chem. 271:19617-24, 1996), purified Hsc70 and human DnaJ homolog Hdj-1 (Hsp 40) were used with additional cofactors provided in reticulocyte lysates (5% v:v) to produce a system capable of refolding denatured luciferase. Briefly, additional cofactors included, recombinant Luciferase (Promega: QuantiLumTM), that had been heat denatured at 42° C for 10 min, 1.8 μ M Hsc70 (Sigma; purified from bovine brain), 0.9 μ M Hsp40, and various recombinant purified proteins.

Luciferase activity was measured (Promega luciferase assay kit) using a luminometer (EG&G Berthold, MicroLumat luminometer, Model #LB96P). All results were normalized relative to non-denatured luciferase that had been subjected to the same conditions. Control reactions lacking ATP, Hsc70, or Hsp40 resulted in negligible luciferase refolding.

On page 37, please delete the paragraph on page 37, line 22, to page 38, line 5, and substitute therefor:

Hip was purified as His₆-protein. The fusion protein was induced from pET28-Hip (V. Prapapanich et al., *Mol Cell Biol.*, 18:944-952, 1998, which is incorporated herein by reference) with 0.1 mM IPTG at 25° C for 6 h in BL21 cells. Cells from 1L of culture were resuspended into 50 ml of 50 mM Phosphate buffer (pH 6.8), 150 mM NaCl, and 1% (v/v) TweenTM-20 and then incubated with 0.5 mg/ml lysozyme at 25° C for 0.5 h, followed by sonication. After centrifugation at 27,500 g for 10 min, the resulting supernatant was mixed with 15 ml nickel resin (Qiagen, Inc.) at 4° C for 3 h with 25 mM imidazol. The resin was then washed with 50 mM phosphate buffer (pH 6.8), 25 mM imidazol, 150 mM NaCl and 0.1% TweenTM-20 until the OD280 nm reached a value of <0.01. His₆-Hip protein was eluted with 250 mM imidazol in washing buffer (Qiagene, Inc.) and purified on Mono QTM (HR10/10 Pharmacia) by FPLC using a linear gradient of 0.5M NaCl at pH 8.0, followed by dialysis in chaperone assay buffer.

On page 45, please delete the first line and substitute therefor:

ABSTRACT OF THE INVENTION